

FACTORS AFFECTING MUSCLE ACTIVATION IN THE HYDROID *TUBULARIA*

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The control of muscle contraction in coelenterates is complex and not well understood. For example, some muscles of sea anemones are activated by a diffuse, all-or-nothing conducting system, probably the nerve net of the column and mesenteries (Pantin, 1935a, 1935b, 1952; Pickens, 1969; Robson and Josephson, 1969). There are requirements for facilitation such that a single impulse in the conducting system initiates little or no mechanical response while a pair of impulses at a suitable interval evokes a moderately rapid twitch. During repetitive stimulation the mechanical response both facilitates and sums. The site of facilitation appears to be between the conducting system and the muscle membrane, probably at the neuromuscular synapse (Pantin, 1935a; Josephson, 1966; Robson and Josephson, 1969). While these features seem conventional, other properties of the muscles are not. The same muscles in anemones which give rapid contractions can also contract very slowly. The slow contractions frequently occur spontaneously and can be slowly propagated along the muscle sheet (Batham and Pantin, 1954; Ewer, 1960; Pantin, 1965a). Conducting systems have been identified which initiate and inhibit slow contractions (McFarlane, 1974) but in general the mechanisms coordinating slow contractions are unclear.

In scyphomedusae, bell contractions are initiated by impulses in an all-or-nothing conducting system, here almost certainly a nerve net (Horridge, 1954). Facilitation is less pronounced in medusae and a long refractory period of the muscle prevents appreciable summation (Bullock, 1943; Horridge, 1955). Twitches of the bell musculature can be augmented by contemporary activity in a second conducting system (Horridge, 1956), but the mechanisms of this augmentation are unknown.

Muscle contraction in hydrozoans has been less studied than in scyphozoans or anthozoans, largely because the small size of most hydrozoans is not conducive to tension measurement. In hydra, where tension recordings are available, longitudinal contraction of the column is peculiarly biphasic (Josephson, 1967). Excitation in a column conducting system initiates a rapid tension increase followed by a slower tension rise. Whether both tension components are generated by the same contractile cells or how either component is initiated is unknown.

Thus coelenterate muscle contraction varies with the pattern of impulses arriving at the muscle; the contraction may be influenced by activity in more than one conducting system; and muscle sheets, possibly even individual muscle cells, may produce both fast and slow contractions (*cf.* Horridge, 1956; Pantin, 1952, 1965b). Details about the mechanisms of muscle activation are obscure. Some of the uncertainties about the control of muscle contraction could be resolved with intracellular recording but the small size and contractility of coelenterate epithelio-

muscular cells make intracellular recording a formidable task. One is left then with less direct measures, developed tension or extracellularly-recorded muscle action potentials, to use in analyzing the mechanisms controlling muscle contraction.

Electrical events which are probably muscle action potentials can be easily recorded from the hydroid *Tubularia* with surface suction electrodes. These potentials can be used to quantify muscle activation without having to directly record muscle tension. This paper considers some of the factors modifying the amplitude of these potentials and hence, by inference, the intensity of muscle activation.

Some background information on the morphological and functional organization of *Tubularia* would be appropriate at this point. *Tubularia* is a moderately large hydrozoan polyp with two sets of tentacles, a proximal set at the base of the hydranth and a distal set which encircles the mouth. The hydranth is borne on a long stalk. In mature polyps gonophores hang from stalks which arise just distal to the proximal tentacles. The spontaneous behavior of *Tubularia* results from the presence of a number of interacting pacemaker systems, the two most important of which are the neck pulse (NP) system in the distal stalk and the hydranth pulse (HP) system in the hydranth (Josephson and Mackie, 1965). The NP system produces electrical pulses (neck pulses = NP's) which typically occur as a sequence of single pulses interrupted periodically by a burst of pulses. Potentials from the HP system (hydranth pulses = HP's) occur as single pulses and pulse bursts. When the HP system fires it initiates coordinated closure of the proximal tentacles and weaker, synchronized closure of the distal tentacles.

Three conducting systems have been identified in *Tubularia* (Josephson, 1965) of which one, the distal opener system (DOS), is of interest here. The DOS courses through the stalk and hydranth. Activating the DOS initiates contraction of the aboral longitudinal musculature of the distal tentacles resulting in outward flaring (opening) of the distal tentacles. Activity in the DOS also inhibits the HP system and other pacemaker systems in the hydranth (Josephson and Uhrich, 1969; Josephson and Rushforth, 1973). Electrical potentials from the DOS can be recorded with surface suction electrodes or extracellular microelectrodes. In the stalk and most of the hydranth, DOS pulses (DOSP's) are small (50–200 μ V), short (9–15 msec), all-or-nothing electrical events. In the vicinity of muscles activated by the DOS a second component appears in the DOSP. With an electrode on a distal tentacle or at the base of the distal tentacles the short, all-or-nothing pulse is followed by a slow, graded potential. This second DOSP component is thought to be a muscle action potential because its amplitude varies monotonically with that of observed tentacle flaring. Both the slow potential and tentacle opening defacilitate to the same degree during repetitive stimulation and both decline in parallel during anesthetization with excess magnesium (Josephson, 1965). The initial DOSP component is unchanged during repetitive stimulation (Fig. 1A) and its amplitude remains constant during anesthetization until it abruptly ceases to appear. Thus the initial DOSP component appears to be generated by the conducting system, the second component by the responding musculature. The amplitude of the second DOSP component is here used as a measure of the intensity of muscle activation.

MATERIALS AND METHODS

Tubularia were collected from the Woods Hole area. Animals were obtained from different places at different times of the year and, judging by the information provided by Miller (1969), included three species; *T. crocea* from the jetty at New Bedford, *T. spectabilis* from Woods Hole and the Cape Cod Canal, and *T. larynx* from the Cape Cod Canal. For the physiological parameters considered in this study, animals thought to be *T. larynx* and *T. spectabilis* were indistinguishable. *T. crocea* differs from the other two in having a longer, more active proboscis, in producing generally larger DOSP's and in other particulars described below.

The stimulating and recording methods are described in detail elsewhere (Josephson and Uhrich, 1969; Josephson and Rushforth, 1973). Briefly, animals were stapled to the bottom of a dish of sea water cooled to 16–18° C. A suction electrode on a gonophore or gonophore stalk was used to activate the DOS. The stimuli were 1 msec voltage pulses. Another suction electrode on the base of a distal tentacle recorded DOSP's and HP's. Recorded activity was displayed on a pen-writer or oscilloscope.

RESULTS

Defacilitation during repetitive DOS activation

The most obvious factor affecting DOSP amplitude is preceding DOS activity; the slow component of DOSP's shows marked defacilitation (Fig. 1A; Josephson, 1965). The time course of defacilitation was examined by stimulating the DOS at a set of interstimulus intervals ranging from 2 to 300 seconds. In each trial the intervals of the set were presented in random order. Results from four animals, each subjected to 20 sets of interstimulus intervals, are shown in Figure 2. In one of these animals the DOSP's reached maximum size with interstimulus

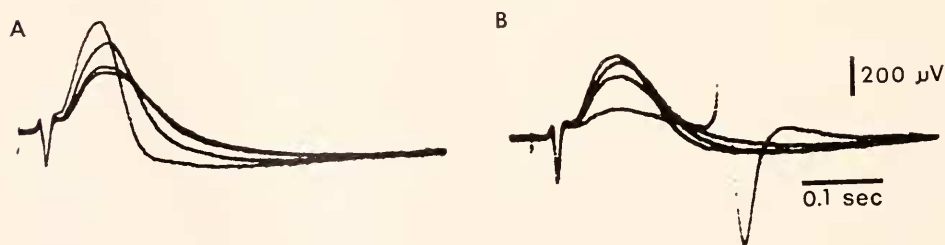


FIGURE 1. Depression of DOSP's during repetitive stimulation and following HP activity; (A.) defacilitation of the second component during stimulation at five-second intervals. The largest response is to the first stimulus which came after a long rest; subsequent responses became progressively smaller. (B.) DOSP's during and after an HP burst; the DOS was being regularly stimulated at 10-second intervals. The first DOSP, the one with the greatly reduced second component, occurred in the burst; the next three with progressively increasing amplitude came 7, 17, and 27 seconds after the burst. The first DOSP was followed by an HP (the potential halfway through the trace) which is poorly resolved at the high amplification used. Positive potentials are upward deflections. Note the constant amplitude of the first component.

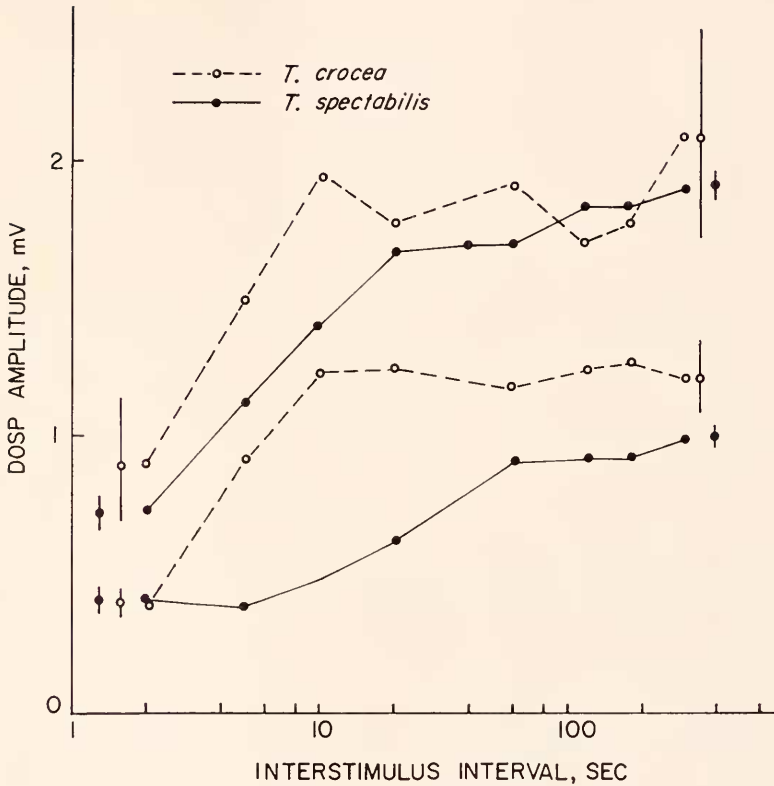


FIGURE 2. Amplitude of the DOSP second component as a function of interstimulus interval. Each point is the average of 20 determinations; standard errors for the first and last points are shown to the left and right of the curves. See text for further information.

intervals of ten seconds or more; in the other three, DOSP's continued to increase with increasing interval to the longest used. In the four animals the DOSP amplitude after a 300-second interval was 2.3 to 3.1 times greater than that with an interstimulus interval of 2 seconds.

The pattern of DOSP amplitude change during bursts of stimuli varies with the stimulus frequency. With interstimulus intervals of about five seconds or more, DOSP's decline monotonically during the burst until they reach an approximate steady state after three or four stimuli. At shorter intervals the DOSP depression sometimes overshoots and the response to the second stimulus may be the smallest of the series, subsequent DOSP's growing slightly until a steady state is reached (Fig. 3).

It might be noted that defacilitating effector responses are unusual in coelenterates; facilitating responses being more typical (*e.g.*, Pantin, 1935a; Bullock, 1943; Morin and Cooke, 1971). Another example of defacilitating muscle contraction, again from a hydroid, occurs in the lashing of dactylozooids from *Hydractinia* (Stokes, 1974).

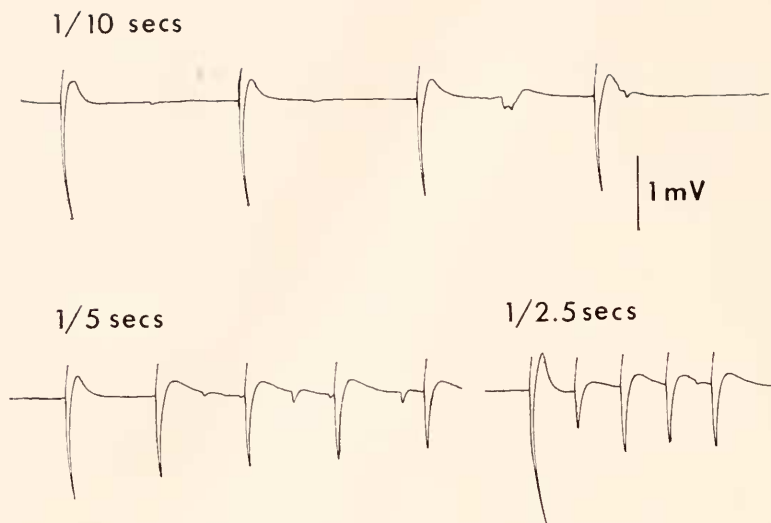


FIGURE 3. Defacilitation of the DOSP during repetitive stimulation, *T. crocea*. The short, upward deflections preceding the DOSP's are stimulus artifacts. In this and the other pen-writer records (Figs. 4, 6) positive potentials are downward deflections.

Depression following pacemaker activity

DOSP's closely following HP's tend to be smaller than those coming long after HP's (Fig. 4). As with defacilitation it is only the second component of the DOSP which is depressed (Fig. 1B). The time course of this depression was examined by stimulating the DOS at a regular frequency so that defacilitation reached a steady state and then determining the DOSP amplitude as a function of the interval between the DOSP and preceding HP activity.

Rather large sample sizes and therefore long recording sessions were required for reasonable temporal resolution. For example, the data of Figure 5A represents over 4 hours of continuous stimulation and recording. During long sessions the amplitude of recorded DOSP's often declines. This may be due in part to fatigue but probably more largely to slippage of the recording electrode or tissue damage beneath the electrode. To compensate for this drift the amplitude of a DOSP following HP firing was expressed as a percent of the DOSP size immediately before the HP activity. The stimulus frequency was one per 10 seconds and the stimuli were initially about twice DOS threshold. During long stimulation periods the DOS occasionally failed to fire following a stimulus. Each time there was such a miss the stimulus intensity was increased. If this eventually did not give faithful following, the stimulating electrode was repositioned and the run continued. Because of partial reduction in defacilitation, the DOSP was larger than normal following the first and sometimes the second successful stimulus after an unsuccessful one. For this reason only those instances were considered in which the control DOSP, that immediately before the HP activity, was preceded

by two consecutive successful stimuli. Similarly if there was a miss following the HP activity, subsequent DOSP's in the post-HP interval were not included in the tabulated results. Another restriction was that the control DOSP had to be separated from the HP preceding it by more than 20 seconds, the major part of the HP effect on DOSP's being over by this time.

Results from the animals giving the two longest series are shown in Figure 5. It is apparent that DOSP depression by HP's is greater following long HP bursts (4 or more pulses) than after short bursts (2 or 3 pulses) and slightly greater after short bursts than following single HP's. Recovery from depression by HP's is at first rapid but there is some indication that recovery can continue for as long as 40 to 60 seconds. At long post-HP intervals the DOSP's are, on the average, larger than controls. This is probably because the DOSP's in these bins occur at post-HP intervals which are longer than is true for the average control DOSP.

HP depression of DOSP's is much more erratic in *T. crocea* than in the other species. In only a few of the more than 20 specimens of *T. crocea* examined was there clear depression and in these the variability in the extent of depression was too great to allow mapping its time course. The depression could be quite obvious. For example, in one *T. crocea*, stimulated at one shock each five seconds, the first DOSP following a single HP averaged only 61% of the amplitude of the DOSP preceding the HP (s.e. = 5%, $n = 28$). The probability of this reduction occurring by chance is extremely low ($P < 0.005$). But this was an exceptional case and more usually depression was absent or too small to be readily detectable.

Depression following spontaneous tentacle activity

Distal tentacles show several kinds of spontaneous activity including oral or aboral movements of one or a few tentacles, simultaneous outward flaring of all

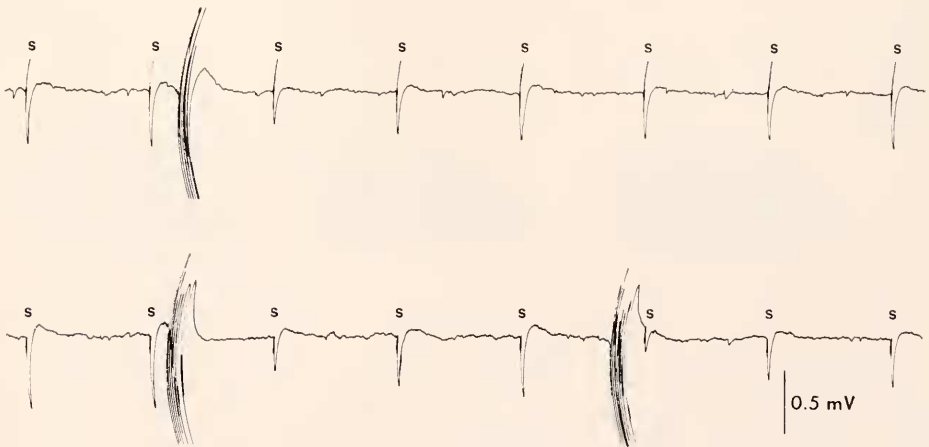


FIGURE 4. Depression of DOSP's following HP bursts. The upper and lower traces are from different parts of a long record during which the DOS was stimulated once each 10 seconds. Stimuli are marked by S in the figure. One HP burst occurs in the top trace, two in the lower trace. Note that DOSP's are depressed following these bursts. The records shown are a portion of those used to construct Figure 5A.

tentacles, and coordinated outward flaring beginning at one point on the tentacle crown and progressing around unidirectionally. The latter will be called a metachronal tentacle flare. Simultaneous outward flaring of all distal tentacles appears to be due to spontaneous firing of the DOS; it is associated with electrical potentials identical to evoked DOSP's. Evoked DOSP's following a spontaneous one are depressed, presumably because of the same defacilitation seen during series of evoked DOSP's.

There are electrical events associated with the other spontaneous tentacle movements as well. Activity in one or a few tentacles is recorded as small, irregular potential deflections; metachronal tentacle flares are recorded as large potentials, a bit smaller and slower than DOSP's and lacking the first DOSP component. The spontaneous tentacle movements and associated potentials are best seen in *T*.

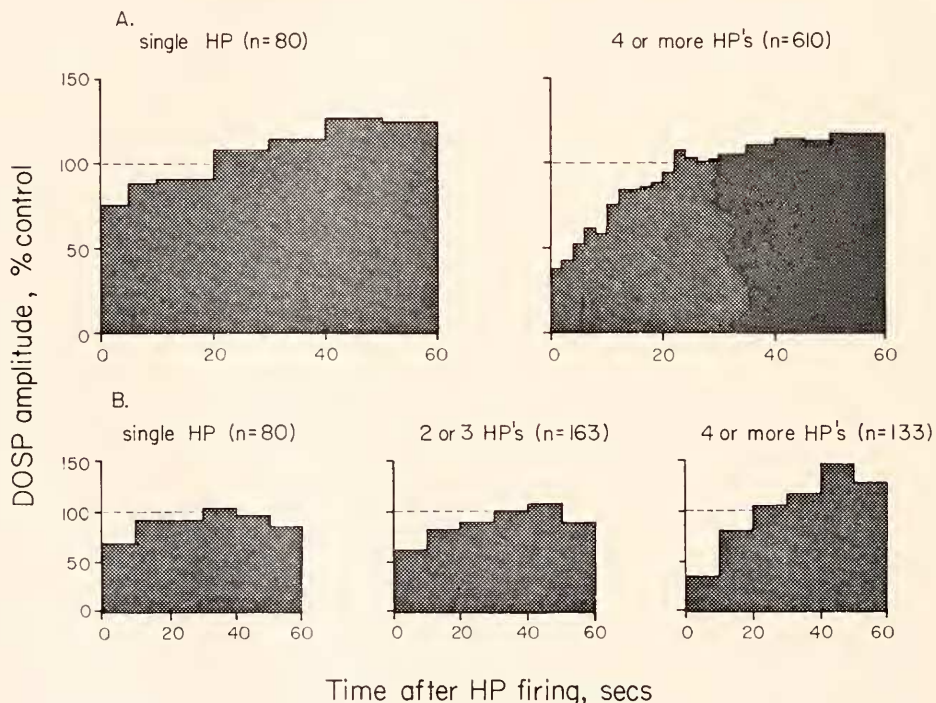


FIGURE 5. DOSP amplitude following HP activity. A is from the animal for which the longest series of records was collected. This animal produced only single HP's and long HP bursts. B is from another animal, one which produced bursts with 2 or 3 pulses as well as longer bursts and single HP's. The intervals between HP activity in these animals ranged from less than 5 seconds to more than a minute. Therefore the number of DOSP's in a given time block decreases with increasing time after HP activity; the longer the post-HP time the fewer the number of HP intervals long enough to contain that time. The bins in the histograms of A have been increased in width at longer post-HP times so that each bin contains approximately the same number of entries. Further explanation is given in the text.

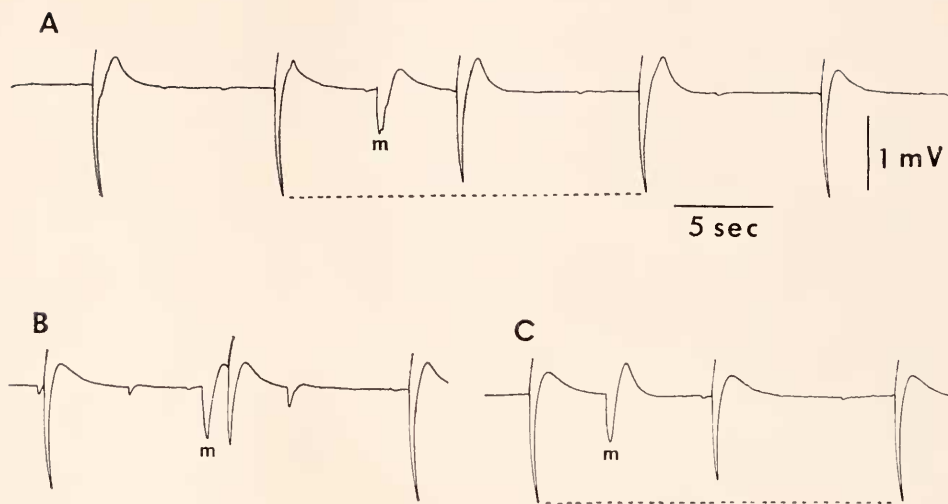


FIGURE 6. DOSP's following spontaneous distal tentacle activity. The events marked m are electrical potentials associated with sequential distal tentacle flaring in a metachronal wave. The dotted lines in A and C connect the DOSP peaks before and two after the spontaneous activity to emphasize the reduction in the DOSP immediately following the metachronal flare.

crocea which has an active proboscis and in which the electrical events are generally larger than in the other species.

DOSP's are depressed following aboral movements of scattered tentacles and particularly following metachronal tentacle flaring (Fig. 6). In the animal of Figure 6 metachronal flares occurred 13 times during periods of regular DOS stimulation at one shock each 10 seconds. The amplitudes of DOSP's immediately following metachronal flares averaged 91% of the DOSP's preceding the flares (s.e. = 2.5%, $P < 0.01$). Because spontaneous distal tentacle activity is of erratic occurrence and variable amplitude, no attempts have been made to quantify the time course of DOSP depression following this activity.

The most complete DOSP depression encountered occurred during defecation. In defecation the mouth slowly opens widely and rolls backwards over the proboscis, turning the proboscis inside out. In three animals, two specimens of *T. crocea* and one *T. spectabilis*, defecation occurred during a recording session without displacing the recording electrode. During the period when the mouth is open and inflected back over the proboscis, the second component of the DOSP is completely suppressed; DOSP's occur as only apparently normal first components.

DISCUSSION

The two components of DOSP's recorded from distal tentacles have different properties and are probably generated by different kinds of cells. The initial component is similar in shape and amplitude to DOSP's recorded elsewhere in the polyp. This component reflects activity in the conducting system, either electro-

genesis by the conducting cells themselves or responses triggered in other cells by the conducted activity (see Ball and Case, 1973, for an example of separable conduction and electrogenesis). For reasons given above the graded second component of the DOSP is considered to be a muscle action potential, presumably generated by membranes of the epitheliomuscular cells which form the opening musculature of the distal tentacles. It was earlier suggested that the DOS is a neuronal conducting system (Josephson, 1965). The clear distinction between the first and second components of the DOSP (*e.g.*, Fig. 1) supports this view, at least it indicates that the DOS is not part of the same epithelial sheet which, in the vicinity of the distal tentacles, forms the opening musculature. Were the DOS an epithelial conducting system one would expect the recorded DOSP to gradually change when it entered the muscle area, perhaps becoming larger as the expanded membrane around the contractile processes became involved. Instead the DOSP is quite similar to that elsewhere but with a new component added after a distinct delay. While certainly not proving the identity of the DOS, the form of the recorded potentials is consistent with the idea that the initial DOS component is neuronal, the slower component from epitheliomuscular cells, and the delay between the two due to transmission processes between the conducting system and the muscle. In fact the delay between the first and second DOSP components is 9 to 20 msec which is sufficiently long that there may be several steps involved in the coupling between the two. This delay is longer than that between nerve net potentials and muscle action potentials in anemones (0–8 msec; Robson and Josephson, 1969; Pickens, 1969); about the same as the delay between conducting system pulses and evoked luminescence in the hydroid *Obelia* (Morin and Cooke, 1971) and considerably shorter than the delay between the onset of electrical activity and that of column contraction in hydra (100–150 msec; Josephson, 1967).

Several factors have been identified which selectively depress the second component of DOSP's: preceding DOS activity, HP firing, spontaneous distal tentacle activity including but not restricted to spontaneous DOSP's, defecation, and partial anesthetization with magnesium (for the last see Josephson, 1965). The recovery of tentacle responsiveness is slow, taking tens of seconds after HP firing and minutes after DOS activity. The constancy of the first DOSP component and the lability of the second indicates that factors affecting DOSP's act on the link between the two components, *i.e.*, they affect transmission processes between the conducting system and the muscle cells. There are several possible sites at which the DOSP reduction could be being effected; the membranes of the muscle cells (post-junctional depression), the terminals of the conducting system (pre-junctional depression) or unidentified elements interposed between the conducting system and the muscle cells. Some consequences of the first two possibilities as sites of depression will now be considered.

Depression of conducting system efficacy

Depression by excess magnesium and defacilitation can be readily interpreted as due to pre-junctional mechanisms. Excess magnesium characteristically blocks transmission at chemical synapses by reducing the amount of transmitter released by presynaptic terminals (*e.g.*, del Castillo and Engbaek, 1954), in turn resulting

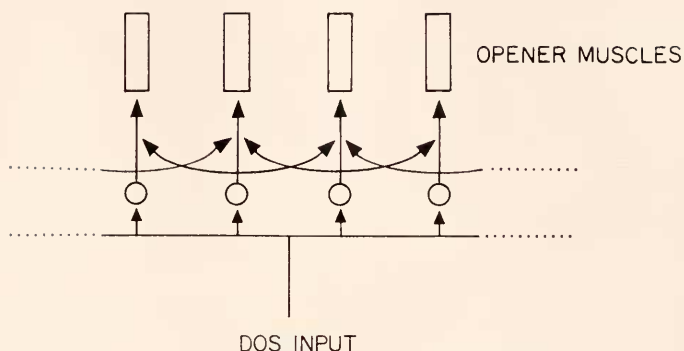


FIGURE 7. A scheme which could account for DOSP depression during repetitive DOS stimulation and following spontaneous distal tentacle activity on the basis of reduced efficacy of shared final terminals. The DOS synchronously activates a set of interconnected elements through polarized junctions. Spontaneous activity spreads laterally through the interconnected elements without exciting the main conducting pathway of the DOS.

in a smaller post-synaptic response. Although there may be both pre-junctional and post-junctional components in synaptic depression, a major factor is often depletion of available transmitter stores (*e.g.*, Thies, 1965). The obvious defacilitation of DOSP's could similarly be due to a reduced pool of available transmitter and hence less transmitter released to later stimuli of a series. The HP effects of DOSP's could also be due to reductions in the amount of transmitter released. The HP depression of DOSP's would then be an example of pre-synaptic inhibition. It is tempting to regard the DOSP reduction following spontaneous tentacle activity as due to the same defacilitation seen during repetitive DOS activation. If these are both pre-junctional effects it would require that spontaneous activity and DOS activation excite the opener muscles through the same final terminals. When activated by the DOS the tentacle opening appears synchronous; during spontaneous activity the tentacles can open sequentially, indicating rather slow lateral propagation. A scheme which would allow synchronous tentacle activation by the DOS and sequential activation during spontaneous activity is shown in Figure 7.

Depressed responsiveness of post-junctional membrane

Many of the features of DOSP depression could also be explained as due to the muscle membrane being partially refractory following activity. The time course of DOSP recovery would then reflect the time course of returning muscle responsiveness. Refractoriness following activity would readily account for the decline of DOSP's during repetitive stimulation and following spontaneous activity, but not for the reduction of DOSP's during magnesium anesthetization since this occurs without muscle activity.

There are at least two ways that HP firing could depress muscle responsiveness. The HP system may directly inhibit the muscle membrane by classical chemical inhibition. Alternatively the depression could again be related to post-firing

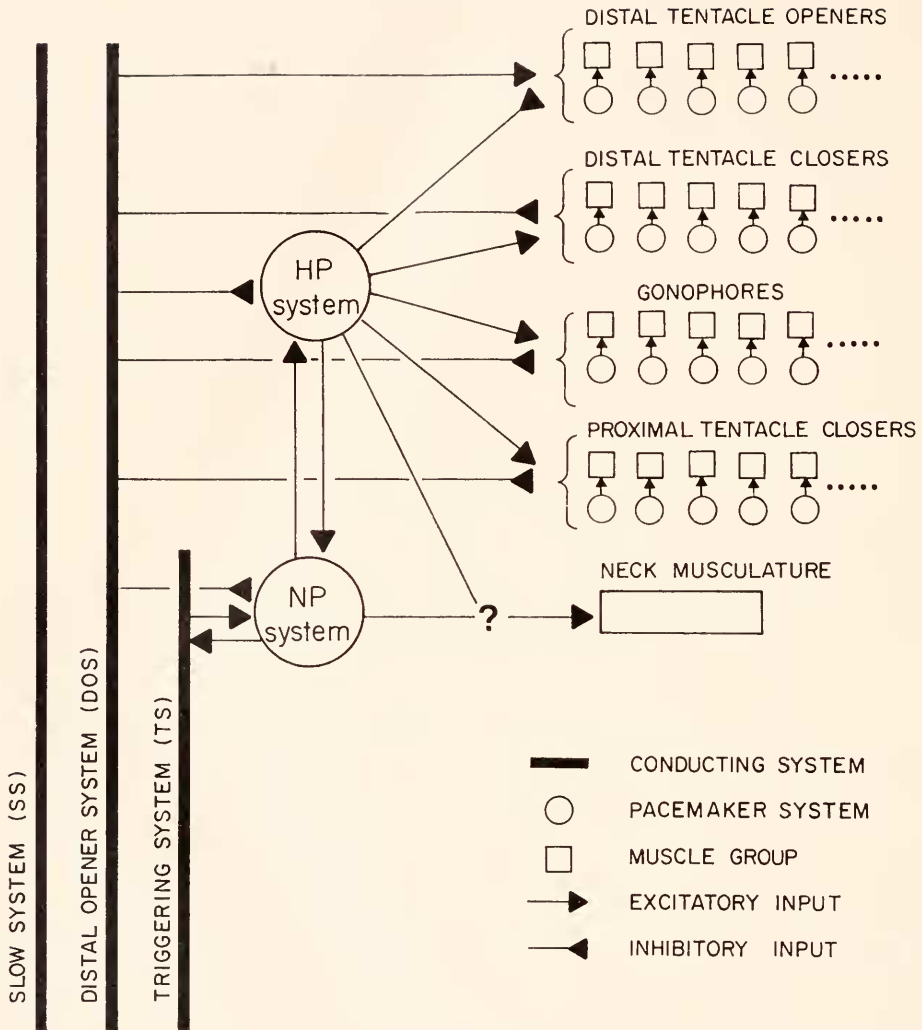


FIGURE 8. The interactions between conducting systems, pacemaker systems and effectors in a *Tubularia* polyp. This figure is based on one published earlier (Josephson and Uhrich, 1969) with the addition of gonophore interactions from unpublished observations of N. B. Rushforth and the inhibition of distal tentacle openers discussed in the text.

refractoriness. HP firing initiates weak tentacle closing, but this does not preclude the possibility that the HP system simultaneously activates the opener muscles and the closing seen is a balance between fully active closer musculature and weaker or only partially-activated opener musculature. It should be stated that there is no evidence for opener muscle activation during HP firing. DOSP's are not recorded from the polyp or stalk during HP firing, indicating that the

conducting system is not excited. The muscle action potentials could be obscured by the usually larger HP's, but in some recordings from *T. crocea* the second DOSP component was larger than HP's and should have been seen during HP firing were it present.

So little is known about the mechanisms of mouth opening and proboscis eversion which are part of defecation that it seems fruitless to try to assign a pre-junctional or a post-junctional site to the DOSP depression during defecation. Certainly the circular muscles around the mouth must relax; if the longitudinal muscles of the distal tentacles are involved in mouth opening or proboscis eversion is just not known.

The above discussion is not meant to imply that all factors affecting DOSP amplitude operate on the conducting system nor that all operate post-junctionally on the muscle membrane. These speculations do emphasize that there are a number of mechanisms which could account for the observed results and they indicate that with available knowledge it is not possible to distinguish which of the many possibilities are correct.

Reciprocal inhibition in Tubularia

From a behavioral viewpoint perhaps the most interesting of the interactions considered is the depression of DOSP's following HP firing. No matter what the mechanism, the net effect is inhibition of tentacle flaring by the HP system. The HP system and the DOS are antagonistic in their effects on the distal tentacles; the former initiating closing and the latter opening of the tentacles. The two systems are also mutually inhibitory although in an unusual way. Activity in the DOS inhibits pacemaker firing by the HP system (Josephson and Uhrich, 1969; Josephson and Rushforth, 1973), and HP activity inhibits the tentacle movements which are a consequence of the DOS activity. These interactions and others previously identified in *Tubularia* are summarized in Figure 8. This diagram emphasizes the complexity of behavioral coordination in *Tubularia*, a complexity which extends to the activation of individual muscle groups.

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SUMMARY

1. Activating the distal opener system (DOS), one of the conducting systems of the hydroid *Tubularia*, causes synchronous opening of the distal tentacles. Simultaneously recorded potentials (DOS pulses = DOSP's) from the vicinity of the responding muscles have two components: (a) an initial, short, all-or-nothing pulse reflecting activity in the conducting system, and, after a brief delay; (b) a

second, slower potential thought to be a muscle action potential because its amplitude varies monotonically with that of tentacle movement. The amplitude of the second component is here used as a measure of the intensity of muscle activation.

2. The second DOSP component is depressed by preceding DOS activity (defacilitation); by spontaneous tentacle movement including but not restricted to spontaneous DOS firing; by firing of the HP system, one of the pacemaker systems in the polyp; and during the mouth opening associated with defecation. In all instances the first DOSP component is unchanged, indicating that the depression is of transmission processes between the conducting system generating the first component and the muscle membrane producing the second component. The recovery from depression is slow, taking tens of seconds following HP activity and minutes after DOS firing.

3. The HP system and the DOS are mutually inhibitory. Previous work has shown that activity in the DOS inhibits HP firing; here it was found that HP firing inhibits the tentacle response which is a consequence of DOS activity. The depression following spontaneous tentacle movements suggests that spontaneous activity and DOS firing may excite the muscles through a common final pathway.

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